Is a mango just a mango? Testing within-fruit oviposition site choice and larval performance of a highly polyphagous fruit fly

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Abstract

For fruit flies, fully ripe fruit is preferred for adult oviposition and is superior for offspring performance over unripe or ripening fruit. Because not all parts of a single fruit ripen simultaneously, the opportunity exists for adult fruit flies to selectively choose riper parts of a fruit for oviposition and such selection, if it occurs, could positively influence offspring performance. Such fine scale host variation is rarely considered in fruit fly ecology, however, especially for polyphagous species which are, by definition, considered to be generalist host users. Here we study the adult oviposition preference/larval performance relationship of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), a highly polyphagous pest species, at the "within-fruit" level to see if such a host use pattern occurs. We recorded the number of oviposition attempts that female flies made into three fruit portions (top, middle and bottom), and larval behavior and development within different fruit portions for ripening (color change) and fully-ripe mango, *Mangifera indica* L. (Anacardiaceae). Results indicate that female *B. dorsalis* do not oviposit uniformly across a mango fruit, but lay most often in the top (i.e., stalk end) of fruit and least in the bottom portion, regardless of ripening stage. There was no evidence of larval feeding site preference or performance (development time, pupal weight, percent pupation) being influenced by fruit portion, within or across the fruit ripening stages. There was, however, a very significant effect on adult emergence rate from pupae, with adult emergence rate from pupae from the bottom of ripening mango being approximately only 50% of the adult emergence rate from the top of ripening fruit, or from both the top and bottom of fully-ripe fruit. Differences in mechanical (firmness) and chemical (total soluble solids, titratable acidity, total non-structural carbohydrates) traits between different fruit portions were correlated with adult fruit utilisation. Our results support a positive adult preference/offspring performance relationship at within-fruit level for *B. dorsalis*. The fine level of host discrimination exhibited by *B. dorsalis* is at odds with the

general perception that, as a polyphagous herbivore, the fly should show very little discrimination in its host use behavior.

Introduction

For many herbivorous insects, selection of oviposition site depends on the quality of the host plant for offspring development (Wilson 1988; DiTommaso and Losey 2003; Van Nouhuys et al. 2003). As a specialist group of insect herbivores, true fruit flies (Diptera: Tephritidae) are also known to make decisions about which fruit to oviposit into based on the suitability of the fruit for their offsprings' performance (Fitt 1981; Joachim-Bravo et al. 2001; Fontellas-Brandalha and Zucoloto 2004). For fruit flies, the quality and amount of nutrients that are available to larvae influence larval size, development time, pupal weight, probability of adult eclosion and reproductive capacity of adult flies (Carey 1984; Krainacker et al. 1987; Bruzzone et al. 1990; Economopoulos et al. 1990; Chang et al. 2000; Kaspi et al. 2002; Woods et al. 2005).

With apparently strong selective pressures to ensure female preference matches offspring performance, theory predicts evolution will lead to increasingly specialized host use (Bernays and Chapman 1994) and this seems to be the pattern in tephritid flies, where narrow hosts ranges are the norm (Fletcher 1989). In fruit flies, however, such theory is not necessarily matched by experimental results where, contrary to the papers cited above, evidence for strong adult preference/offspring performance relationships is weak (Díaz-Fleischer, et al. 2001). Also contrary to standard host-range theory, polyphagy appears to be a derived, rather than ancestral trait in fruit flies (Díaz-Fleischer, et al. 2001; Graham et al. 2006). Walter (2003) has argued that herbivory theory which focuses on classifying organisms using generic terms such as 'specialist" or "generalist", monophagous or polyphagous, can mislead research by moving the focus of investigation away from the functional interactions which occur between a herbivore and its host plant. Given the discrepancy between theoretical predictions and experimental observation in fruit flies, Walter's argument that we focus

greater attention on the individual interactions between herbivores and their host plants is clearly pertinent in this system.

One example of ignoring individual interactions, common across nearly all fruit fly host use studies, is the treating of individual fruit as homogenous resources. Specifically, while there has been quite extensive work in fruit flies concerning adult preference/offspring performance relationships at the "between-fruit" level (i.e., between different fruit species or different varieties of the one species) (Fitt 1981; Peck and McQuate 2004; Thomas 2004; Balagawi et al. 2005; Navrozidis and Tzanakakis 2005), significantly less has been done at the "within-fruit" level. Fruit fly maggots do not move between fruit during their larval stages and so they need to make up a complete diet from within the host fruit that eggs are placed. In arena situations, larvae of Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), selectively moved to nutritionally superior diets, suggesting that larvae have the capacity to move within fruit to maximize their nutritional intakes (Zucoloto 1987; Zucoloto 1991; Fernandez-Da-Silva and Zucoloto 1993). Similarly, larvae derived from wild caught *C. capitata* showed a strong preference for papaya compared to an artificial diet in an arena (Joachim-Bravo and Zucoloto 1998), again reinforcing the point that, at least for that species, larvae have to capacity to detect and respond to host material of different quality.

Why might pulp within a single piece of fruit be of nutritionally different quality? Firstly, larvae themselves may change host quality, both positively and detrimentally through direct feeding, production of metabolic heat and transfer of bacteria, and there is evidence this occurs (Zucoloto 1987; Zucoloto 1991; Fernandez-Da-Silva and Zucoloto 1993; Joachim-Bravo and Zucoloto 1998; Diaz-Fleischer and Aluja 2003). Another mechanism, and the one pursued in this paper, relates to host ripening. Fruit flies are known to preferentially

92 oviposit into ripe over unripe fruit (Seo et al. 1982; Messina and Jones 1990; Jang and Light
93 1991; Messina et al. 1991; Vargas et al. 1995), while larvae perform better in ripe fruit
94 (Joachim-Bravo et al. 2001; Fontellas-Brandalha and Zucoloto 2004). Better performance in
95 ripe fruit may be due to nutritional status (e.g., higher sugar and lower starch levels) (Bidwell
96 1979; Medlicott and Thompson 1985), but may also be due to changes in allelochemicals. In
97 the Anacardiaceae (which includes mangoes, the focus of this paper), phenolics, resins,
98 alkaloids, saponin and volatile oils play a role in defending plants against phytophagous
99 insects (Keil et al. 1946; Joel 1978; Herrera 1982). Many of these secondary chemicals tend
100 to decrease in concentration as fruit ripens (Macheix et al. 1990). For fruit which ripens
101 gradually (i.e. most climacteric fruit; Bidwell 1979), it is highly likely that some portions of a
102 fruit will be riper, and hence may be nutritionally superior or contain lower levels of
103 allelochemicals, than other portions. In such cases fruit fly larvae may well move themselves
104 to superior sites, or adults may preferentially oviposit into them.
105
106 While studying the influence of mango, *Mangifera indica* L. (Anacardiaceae),
107 ripening on oviposition preference and larval performance for the Oriental fruit fly,
108 *Bactrocera dorsalis* (Hendel), at the "between-fruit" level (Rattanapun et al. 2009), we noted
109 that adult oviposition site selection at the within-fruit level did not appear random. Rather,
110 certain portions of fruit, especially the top, appeared preferred. Whether this was related to
111 differences in fruit quality, with the potential to impact on larval performance, was not clear.
112 To take these observations further we carried out structured laboratory observations to
113 determine: *B. dorsalis* oviposition site preference between the top, middle and bottom
114 portions of mango; larval performance in the top or bottom half of mango; and larval feeding
115 site preference (as judged by larval movement away from different egg insertion points). We
116 concurrently measured fruit paramaters, which may influence larval behavior and survival, at

the same within-fruit scale. All work was carried out on two ripening stages [color change (= mature but still ripening) and fully-ripe] of a commercially produced Thai mango, mango variety Namdorkmai. To be consistent with our usage in Rattanapun et al. (2009), in this paper we will refer to the two ripening stages as “ripe” and “fully-ripe”. Our specific aims were, for *B. dorsalis*, to: (i) determine if there was a positive adult oviposition preference/larval performance relationship at the “within-fruit” level; (ii) determine if larval movement occurred and if so was consistent with a pattern that would be expected if larvae were moving to areas of riper fruit; and (iii) better understand the evolution of polyphagy in this fly.

Materials and Methods

Location

All research was carried out in the laboratory at the National Biological Control Research Center, Headquarters, Kasetsart University, Bangkok, Thailand. Average humidity, temperature and light intensity within the laboratory were 61%, 25 ºC and 331 Lux, respectively.

Eggs and Adult flies

Bactrocera dorsalis were originally obtained from the Department of Agriculture, Bang Khen, Bangkok. The number of generations for which they had been in culture was unknown, and our culture was started with relatively few flies. Adult flies were fed with water and sugar: yeast hydrolysate (3: 1) and larvae were reared on *Musa x paradisiaca*, ABB Group (Musaceae), Namwa variety. The culture was nine generations old when used in trials. To confirm that culturing had not altered the behavior of flies (at least with respect to our questions), a subset of the preference/performance trials was repeated using F1 flies from the

field after laboratory studies had been completed. These trials showed no obvious difference to the patterns of host use shown by cultured flies. Results of the validation trial are available on request from the contact author. Voucher specimens of flies used in the trials are deposited with the National Biological Control Research Center, Headquarters and Department of Entomology, Kasetsart University, Bangkok, Thailand.

Fruit host

Mango variety Namdorkmai of two ripening stages was used to determine the oviposition preference of *B. dorsalis* female flies and preference and performance of larvae for different fruit portions. All fruits were bought from local markets at ripe (green-yellow; marketable after shipment) and fully-ripe (yellow; marketable for local use at the production site) stages. Based on discussion with the fruit sellers (who were also the growers), all fruit purchased had been protected from fly during production by use of fruit bagging, rather than insecticides. To check for possible field infestation of the fruit, in every experiment five mangoes were randomly selected and incubated in separate plastic containers to check for pupal emergence. In total 60 fruits were screened and no pupae were recovered from such controls. In other trials (Rattanapun et al. 2009) we also studied preference and performance of *B. dorsalis* on mango at the mature green stage ripening stage, but oviposition into such fruit was negligible (in both choice and no choice trials) and so we did not use this age class of fruit in the current study.

Fruit properties

All fruits used for fruit property measurements were randomly selected from fruits purchased for behavioral tests, before any fruits had been assigned to experiments. Fifteen fruits from both mango ripening stages were used for measurements of total soluble solids (TSS) (=

Brix), measured using a handheld Brix refractometer (OPTIK B-32; ATAGO, Saitama, Japan). Brix degree is used to measure the sugar, organic acid and other components in the juice of fruit (Linskens and Jackson 1995). Firmness was measured using a penetrometer (FT-327, Effegi, Alfonsine, Italy) with 1 mm diameter probe (as used by Balagawi et al. 2005 and Diaz-Fleischer and Aluja 2003), mounted on a Black & Decker® test stand (Black & Decker, Berkshire, United Kingdom) on each of 13 ripe and fully-ripe mangoes. Thirteen penetrometer readings were taken at three different locations on each fruit portion and averaged for the position. The diameter of the oviposition hole made by female *B. dorsalis* is 0.2 ± 0.01 mm (n = 30, authors' unpublished data). Six ripe and fully-ripe mangoes were also tested for percentage titratable acidity (TA) (following the approach of Hulme 1971) and total non-structural carbohydrates (TNC) [following the acid extraction of Smith et al. (1964) and Nelson's reducing sugar procedure of Hodge and Hofreiter (1962)].

Oviposition site preference, no-choice trial

To evaluate oviposition site preference of *B. dorsalis* females within mangoes of different ripening stage, fruit of the two ripening stages were placed individually into 30 × 30 × 30 cm Perspex observation cages. The place of attachment between the fruit and the mango stem was covered with tape, as preliminary observations showed that female flies preferred this site for oviposition when the site was exposed, despite it not being available to the flies when they attack fruit on the tree. An individual 21- to 22-day-old, mated female fly was released into the observation cage with one mango per replicate. The mango was placed on the center of the cage floor. Twenty single-fly replicates were conducted for each ripening stage and we recorded the number of attempted ovipositions in each of three fruit portions; the top (closest to stem), middle and bottom. While flies actively engaged in oviposition behavior on fruit, almost no successful penetration occurred (an issue discussed by Rattanapun et al. 2009),

thus all results refer to oviposition attempts. Observations were made continuously from 7: 00-17: 00 hours. At the end of the day, flies were dissected to confirm their gravid status: in all cases there were mature eggs in the ovaries.

Oviposition site preference, choice trial

A choice experiment was conducted to determine the behavior of individual female *B. dorsalis* when the two ripening stages were offered simultaneously in a 50 × 50 × 50 cm laboratory cage. Ripe and fully-ripe mangoes were placed at each corner of the laboratory cage and the female fly was released at the equal distance between two mangoes. With the exception of simultaneous offering of fruit, all other experimental conditions were as for the no-choice trial.

Preference of *B. dorsalis* larvae for different fruit portions

Bactrocera dorsalis eggs were collected using an inverted perforated plastic cup swabbed with the flesh of *M. x paradisiaca*. Eggs were placed in water and those which sunk were collected for use: floating eggs are inviable (Balagawi et al. 2005). Using a sterile blade a narrow slit was made in the mango skin near either the top or bottom of the fruit and 20 eggs were inserted using a brush. The mangoes were held for five days and on the fifth day fruit was divided into four portions and larvae in each portion counted. Division of fruit for larval counts was done as follows. Fruit was first halved (by length) and then the fruit half where eggs were inserted was further equally divided into three (again by length). Numbering of portions from one to four began from the portion where eggs were inserted (i.e., when eggs were inserted at the top of fruit then the top-most portion was one and bottom portion four, when eggs were inserted at the bottom of the fruit so the bottom-most portion was one and the top portion four). Division of fruit in this way gave greater sensitivity in assessing larval

movement from the point of egg insertion. Ten replicates for each of eggs inserted at the top and bottom of both ripe and fully-ripe mango were undertaken.

The performance of *B. dorsalis* larvae on different fruit portions

Using the same technique as described above, 20 *B. dorsalis* eggs were inoculated into either the top or bottom of ripe or fully ripe fruit (10 replicates of each). Mangoes were then individually incubated over sand and emergent pupae counted, weighed and left in plastic containers for adult eclosion, when the number of emergent adults was counted and wing length measured (from wing base to wing tip). Wing size is a commonly used measure of adult size in fruit flies (Yuval et al. 1998; Kaspi et al. 2000; Kaspi and Parrella 2003).

Statistical analyses

Results for no-choice and choice oviposition trials were analysed using two-way ANOVA to test for effect of ripening stage and fruit portion. For larval feeding site preference, while the data were amenable to analysis using a single three-way ANOVA (i.e., independent variables ripening class, egg placement, fruit portion), we did not use this analysis because of the inherent difficulties in interpreting third-order interactions. Rather, we investigated interaction effects using four, two-way ANOVAs. We ran two, two-way ANOVAs to test for differences in larval location depending on where eggs were initially placed within a ripening class [i.e., independent variables, egg placement (top/bottom) and fruit portion (1-4); dependent variable, number of larvae; separate two-way ANOVA for each ripening class] and then a further two, two-way ANOVAs to test for differences in larval location when eggs were placed in the same location (either top or bottom) across ripening classes [i.e., independent variables, ripening stage (ripe/fully-ripe) and fruit portion (1-4); dependent variable, number of larvae; separate two-way ANOVA for eggs placed at top or bottom of

fruit]. Because no significant interactions were found in these analyses (see Results), we present the results graphically as simple mean larval abundance in the four fruit portions for each of the four treatments (i.e., ripe or fully ripe fruit, eggs inserted in top or bottom of fruit). For all ANOVAs, *post-hoc*, pairwise comparisons of means was made using Tukey's-test. Independent-samples t-test was used to analyze all parameters of larval performance. Paired-samples t-test was used to determine the different of percentage of TA and TNC content between top and bottom portions. Response variables analyzed were the number of attempted ovipositions, the number of pupae, the weight of pupae, percent adult emergence, the duration of the egg to adult period, wing length and the physical characteristics of mango fruit (i.e., firmness, TSS, TA and TNC). Data were transformed using log (n+1), if required, to meet the assumptions of statistical analysis and then back-transformed for presentation in graphs and tables.

Results

Fruit properties

TSS did not differ significantly between the top, middle or bottom of ripe (ANOVA: $F_{2,42}$ = 0.564, P = 0.573) and fully-ripe mangoes (ANOVA: $F_{2,42}$ = 1.478, P = 0.240). There were significant differences in the firmness among the three fruit portions of ripe mango (the top was softest, ANOVA: $F_{2,36}$ = 30.886, P < 0.0001), while the firmness did not differ significantly among three fruit portions of fully-ripe mango (ANOVA: $F_{2,36}$ = 0.026, P = 0.975). The TA percentage did not differ significantly among top and bottom portions of ripe mango (Paired-samples t-test: t = 2.254, d.f. = 5, P = 0.074), however, there was a significant difference in the TNC content among top and bottom portions of ripe mango (the top had higher TNC, Paired-samples t-test: t = -5.966, d.f. = 5, P = 0.002). For fully-ripe mango, the percentage of TA and TNC contents of both portions did not differ significantly (Paired-

samples t-test: t = 1.222, d.f. = 5, P = 0.276; t = 0.090, d.f. = 5, P = 0.931, respectively) (Table 1).

Oviposition site preference, no-choice trial

Two-way ANOVA did not detect a significant effect of mango ripening stage on oviposition site preference (ANOVA: $F_{1,114}$ = 0.176, P = 0.676), nor was there a significant interaction between mango ripening stage and fruit portion on oviposition site preference (ANOVA: $F_{2,114}$ = 0.859, P = 0.426). There was, however, a significant effect of fruit portion on oviposition site preference. Female flies made significantly more oviposition attempts into the top third of the fruit than the middle third, which was again significantly greater than the bottom third (ANOVA: $F_{2,114}$ = 27.349, P < 0.0001) (Figure 1).

Oviposition site preference, choice trial

Results from the choice trial were identical to the no-choice trial. There was no significant effect of mango ripening stage (ANOVA: $F_{1,114}$ = 0.728, P = 0.395), nor was there a significant interaction between mango ripening stage and fruit portion on oviposition site preference (ANOVA: $F_{2,114}$ = 0.751, P = 0.474). There was again, however, a significant location affect. Female flies again made significantly more oviposition attempts into the top third of the fruit than the middle third, which was again significantly greater than the bottom third (ANOVA: $F_{2,114}$ = 34.135, P < 0.0001) (Figure 1).

Preference of *B. dorsalis* larvae for different fruit portions

Two-way ANOVA detected no significant interaction between initial egg insertion point and infestation level of different fruit portions for ripe (ANOVA: $F_{3,72}$ = 0.772, P = 0.513) or full-ripe mangoes (ANOVA: $F_{2,73}$ = 1.519, P = 0.226). Nor, when comparing across

fruit ripening classes, was there a significant interaction between infestation level of different fruit portions and fruit ripening class when eggs were initially inserted at the top of the fruit (ANOVA: $F_{3,72} = 1.920$, $P = 0.134$), or at the bottom of the fruit (ANOVA: $F_{3,72} = 1.174$, $P = 0.326$).

When eggs were inserted into the top of ripe mangoes, the number of larvae in fruit portion 1 (i.e., the top-most portion) was significantly higher than the third and fourth portions, while the larval number in fruit portion 2 was intermediate between the first and the third portions. The larval number in fruit portion 4 was significantly lower than for all other segments (ANOVA: $F_{3,36} = 15.574$, $P < 0.0001$, Figure 2A). For ripe mangoes where eggs were inserted into the bottom of fruit, the larval number in the first (i.e., bottom most) and second portions were higher significantly than the fourth portion, while the number of larvae of the third portion was intermediate between the two (ANOVA: $F_{3,36} = 6.441$, $P = 0.001$, Figure 2B).

For fully-ripe mangoes with eggs inserted into the top of fruit, the number of larvae did not differ significantly between the first, second and third fruit portions, but each was significantly greater than the number in fourth portion (ANOVA: $F_{3,36} = 9.036$, $P < 0.0001$, Figure 2C). For fully-ripe mangoes where eggs were inserted into the bottom of fruit, the larval number in the first portion was significantly greater than in the fourth portion, while the number of larvae in the second and third portions were intermediate between the two (ANOVA: $F_{3,36} = 3.075$, $P = 0.040$, Figure 2D).

The performance of *B. dorsalis* larvae on different fruit portions

316 Checking of fruit after larvae had pupated indicated that there was no evidence (by way of
317 feeding sites or tunneling) of larvae having left the fruit half where eggs were initially
318 deposited. We therefore had confidence to analyze the data as larval performance in the top
319 half versus the bottom half of fruit.
320
321 For ripe mango, there were no statistical differences between the top and bottom
322 halves of fruit in the average duration of the larval period (Independent-samples t-test: t =
323 0.550, d.f. = 158.500, P = 0.583), percentage pupal recovery (Independent-samples t-test: t =
324 1.832, d.f. = 10.241, P = 0.096), pupal weight (Independent-samples t-test: t = 0.816, d.f. =
325 12.779, P = 0.429), pupal period (Independent-samples t-test: t = 1.082, d.f. = 135, P =
326 0.281), male wing length (Independent-samples t-test: t = -0.259, d.f. = 61, P = 0.796) and
327 female wing length (Independent-samples t-test: t = -0.799, d.f. = 42.343, P = 0.429), but the
328 percentage of adult emergence differed significantly (Independent-samples t-test: t = 2.830,
329 d.f. = 9.189, P = 0.019) (Table 2).
330
331 For fully-ripe mango, there were no statistical differences between the top and bottom
332 halves of fruit in all parameters of larval performance measurement [average duration of the
333 larval period (Independent-samples t-test: t = 1.110, d.f. = 189.409, P = 0.268), percentage of
334 pupal recovery (Independent-samples t-test: t = -0.303, d.f. = 18, P = 0.766), pupal weight
335 (Independent-samples t-test: t = -0.107, d.f. = 18, P = 0.916), pupal period (Independent-
336 samples t-test: t = 0.327, d.f. = 137, P = 0.744), percentage of adult emergence (Independent-
337 samples t-test: t = 0.751, d.f. = 18, P = 0.462), male wing length (Independent-samples t-test:
338 t = -1.160, d.f. = 54, P = 0.251) and female wing length (Independent-samples t-test: t =
339 1.987, d.f. = 81, P = 0.050)] (Table 2).
340

Discussion

Oviposition site preference

Results indicated that female *B. dorsalis'* preferred oviposition site was the top of ripe and fully-ripe mangoes (Figure 1). The oviposition site preference of female flies for the top portion of mango may be partially related with the physiological changes of mango ripening. The top portion of mango fruit ripens earlier than the middle and the bottom, and thus has a softer pericarp than the other portions (at least for ripening fruit) (Table 1). Firmness is considered to be a limiting factor for oviposition of female fruit flies (Seo et al. 1982; Messina and Jones 1990; Balagawi et al. 2005) and is possibly influencing adult preference in the *B. dorsalis* / mango system. We do note, however, that in this study we report only the fruit characteristic of firmness and TSS as possibly factors influencing oviposition site selection. In the field other factors such as fruit volatiles (Jang and Light 1991), wounds or cracks in the fruits (Papaj et al. 1989), oviposition holes of conspecifics (Papaj and Alonso-Pimentel 1997), variation in available water, farming practices and plant diseases (Greany et al. 1985; Liquido et al. 1995; Aluja et al. 2004) may all influence female oviposition preference.

The preference and performance of *B. dorsalis* larvae on different fruit portions

For nearly all data, there was no evidence of larval preference or performance being influenced by different fruit portion, within or across fruit ripening stages. Two-way ANOVA failed to detect any interaction between larval position and either egg insertion point or fruit ripening stage, while visual presentation of results (Figure 2) show a generally common pattern of larvae being in highest density at or near the egg insertion point, becoming less common at greater distances away from that point: normal point dispersal would account for this dispersion pattern. Nearly all measures of larval performance were not significantly

366 different between larvae developing in the top or bottom of ripe and fully-ripe mangoes,
367 again reinforcing the lack of obvious within-fruit effects.
368
369 One very dramatic difference did occur, however, for larvae developing in ripening
370 fruit. Adult emergence from pupae derived from larvae which developed in the bottom half of
371 ripe fruit was only half of that for corresponding pupae from the top of ripe fruit, or for pupae
372 developed from the top or bottom of fully-ripe fruit. If host quality influenced this result then
373 it did not show up in other parameters of larval quality, but would be consistent with other
374 research that has demonstrated that the quality of nutrients that larvae have fed on influence
375 emergence of the adult fruit fly (Economopoulos et al. 1990; Fernandes-da-Silva and
376 Zucoloto 1993; Chang et al. 2000). Significantly lower TNC levels and higher acidity levels
377 in the bottom half of ripe mango (Table 1) may be causal, or at least correlated, with this
378 reduced adult emergence rate.
379
380 The original aims of the paper were to: (i) determine if there was a positive adult
381 oviposition preference/larval performance relationship at the “within-fruit” level; and (ii)
382 determine if larval movement occurred and if so was consistent with a pattern that would be
383 expected if larvae were moving to areas of riper fruit. The second aim appears to have been
384 fully addressed. While some larval movement occurs, it is not consistent with an expectation
385 that larvae should relocate themselves to the ripest (i.e., top most) portion of the fruit.
386 Resolution of the first aim is less clear, but possibly answered in the affirmative. Adults
387 clearly prefer to oviposit in the top of fruit, but for one parameter only (from seven
388 parameters of larval performance measured) was the top of the fruit better for offspring. That
389 one parameter, adult emergence from pupae was, however, quite dramatically different with a
390 50% reduction in adult emergence from pupae derived from the lower half of fruit. When

only one (or few) parameters within a series show a result different to the common trend, it is appropriate to be cautious about interpreting that result in case it is due to chance or unknown experimental error. If, however, the result of high pupal mortality for larvae from slightly under-ripe fruit is real and consistent, then it would explain the preference by the adult for the top of the fruit, as there would be strong selection pressure on the adult to oviposit in sites which are best for offspring development. Mortality of pupae prior to adult emergence strongly suggests that some key chemical component of the fruit is either missing, or existing at toxic levels, and is worthy of further investigation.

Adult oviposition preference may, however, have nothing to do with offspring performance. Fruit flies are well documented as preferring hosts with softer skins and/or flesh (Seo et al. 1982; Messina and Jones 1990; Messina and Jones 1991; Balagawi et al. 2005; Rattanapun et al. 2009). Preference for the top of fruit as an oviposition site may thus be a direct mechanical, or longer-term evolved response, to the fact that a host fruit is, or likely to be, softer at the top. Further research is required to determine which of these two hypotheses (i.e. a positive preference/performance relationship or mechanical suitability) is correct.

Implications for evolution of host use in tephritids

Polyphagous insects are commonly considered generalist users of a wide array of resource types (Walter 2003). Such views are reinforced by published host lists (e.g.Hancock et al. 2000), where listing of a host plant is rarely supported by any biological data which may give insights into how frequently a host is used, or if a host is more or less preferred in comparison to other hosts. For *B. dorsalis*, Allwood et al. (1999) record 124 larval hosts and, as such, the fly is regarded as a highly polyphagous. Despite this tag, however, *B. dorsalis* is known to discriminate between hosts in the lab and field (Clarke *et al.* 2005). For example, based on

field surveys in Thailand, Clarke *et al*. (2001) showed that *B. dorsalis* was quite discriminatory in its host use, with only a small number of the total pool of locally available host plants yielding the greater majority of locally reared flies. When combined with the findings of this paper, that host use varies at the within fruit level, the accumulating results suggest that for even as polyphagous an insect as *B. dorsalis*, a relatively small range of host plant attributes may be involved in host acceptance and/or utilisation. What these attributes may be is as yet unknown, but the recent findings of up to six cryptic species of tephritine feeding within the flower heads of a single daisy species (Condon et al. 2008) suggests an extraordinary ability of tephtritids to detect subtle host differences. This ability may have implications for speciation in this highly diverse family, where there is increasing evidence for host associated cryptic species (Abrey et al. 2005; Stireman et al. 2005; Knio et al. 2007a,b; Marsteller et al. 2009; Smith et al. 2009). We suggest that further research on host use by fruit flies focus on understanding the mechanisms of host utilization, rather than simply documenting the size of the host range.

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Figure legends

Figure 1 The mean (± SE) number of attempted ovipositions by gravid female *Bactrocera dorsalis* into three fruit portions of mango variety Namdorkmai in no-choice and choice trials. The data presented for each trial are pooled from observations made independently on two different ripening stages (n = 40). The *Post-hoc* significance indicators are based on the unpooled data in a 2-way ANOVA.

Figure 2 The mean (± SE) number of *Bactrocera dorsalis* larvae in different fruit portions of mango variety Namdorkmai, six days after 20 egg cohorts were inoculated into either the top or bottom of mango fruit. (A) Ripe mango with eggs placed at the top of fruit; (B) Ripe mango with eggs placed at the bottom of fruit; (C) Fully-ripe mango with eggs placed at the top of fruit; (D) Fully-ripe mango with eggs placed at the bottom of fruit. Numbering of the four fruit portions begins at the fruit end where eggs were inserted. Portion numbers 1-3 equally occupy one-half of a piece of fruit, portion four is the second half. n = 10, 20 egg replicates per treatment.

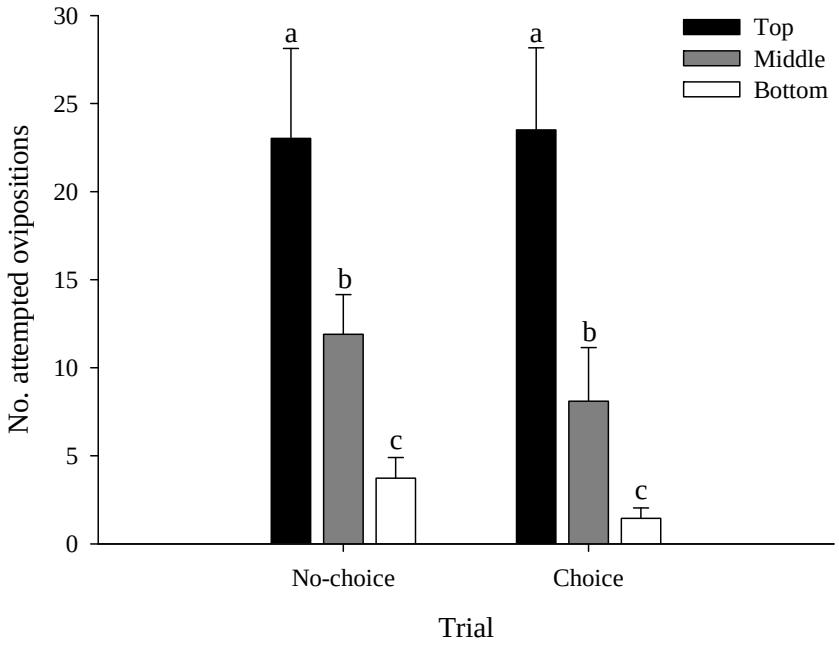
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Figure 1

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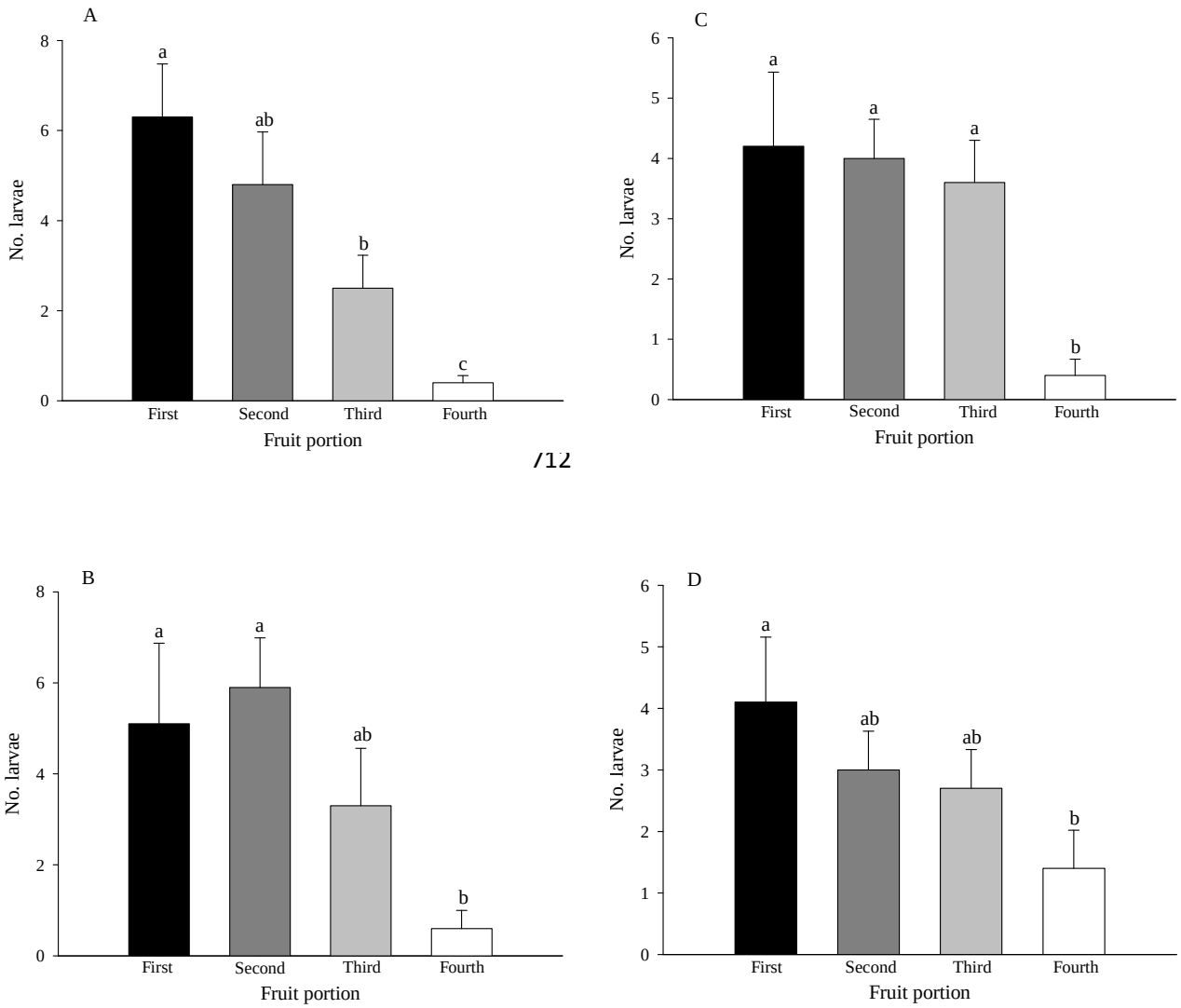

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714 **Figure 2**

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Table 1 The fruit properties of mango variety Namdorkmai at two ripening stages. [n = number of replicates; Values (mean ± SE) in the same column of each mango ripening stage followed by a different letter are statistically different based on Tukey-test for TSS and firmness and Paired-samples t-test for TA and TNC at $P < 0.05$. Significance is based on transformed data using log (x + 1), non-transformed data are presented.]

	TSS (°Brix)	Firmness (kg/cm^2)	TA (%)	TNC (mg D-glucose/g dry weight)
Ripe				
top	15.31 ± 0.31a	0.58 ± 0.03c	0.80 ± 0.16a	125.64 ± 11.82a
middle	15.05 ± 0.31a	0.95 ± 0.03a	-	-
bottom	14.85 ± 0.30a	0.79 ± 0.04b	0.99 ± 0.17a	112.54 ± 10.85b
n	15	13	6	6
Fully-ripe				
top	19.86 ± 0.96a	0.22 ± 0.01a	0.14 ± 0.03a	127.46 ± 3.16a
middle	18.34 ± 0.92a	0.22 ± 0.01a	-	-
bottom	17.73 ± 0.87a	0.22 ± 0.01a	0.15 ± 0.02a	128.00 ± 3.88a
n	15	13	6	6

Table 2 The performance of *Bactrocera dorsalis* larvae developed in different fruit portions of two ripening stages of mango variety Namdorkmai. [n = number of replicates. Each replicate was initiated as a cohort of 20 eggs per fruit stage. Values (mean ± SE) in the same column of each mango ripening stages not followed by the same letter are significantly different based on Independent-samples t-test at $P < 0.05$. Significance is based on transformed data using log (x + 1), non-transformed data are presented.]

Mango ripening stages / fruit portion	Larval period (days)	Pupal recovery (%)	Pupal weight (g)	Pupal period (days)	Adult emergence (%)	Wing length (mm)	
						male	female
ripe							
top (n = 10)	11.64 ± 0.33a	56.50 ± 6.67a	0.158 ± 0.017a	10.13 ± 0.16a	73.57 ± 4.48a	6.04 ± 0.06a	6.19 ± 0.03a
bottom (n = 10)	11.70 ± 0.47a	45.50 ± 12.68a	0.129 ± 0.035a	9.88 ± 0.13a	35.13 ± 11.10b	6.06 ± 0.03a	6.24 ± 0.04a
fully-ripe							
top (n = 10)	12.80 ± 0.55a	52.00 ± 5.97a	0.138 ± 0.018a	10.43 ± 0.11a	69.10 ± 7.04a	6.08 ± 0.04a	6.27 ± 0.03a
bottom (n = 10)	11.83 ± 0.46a	53.00 ± 4.36a	0.140 ± 0.011a	10.41 ± 0.17a	61.26 ± 6.18a	6.13 ± 0.03a	6.19 ± 0.03a